Predatory Organisms with Untapped Biosynthetic Potential

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Predatory organisms with untapped biosynthetic potential. A description of eight novel C*orallococcus species*: *Corallococcus aberystwythiensis* sp. nov., *Corallococcus carmarthensis* sp. nov., *Corallococcus exercitus* sp. nov., *Corallococcus interemptor* sp. nov., *Corallococcus llansteffanensis* sp. nov., *Corallococcus praedator* sp. nov., *Corallococcus sicarius* sp. nov., and *Corallococcus terminator* sp. nov.

Paul G. Livingstone[1,2], Oliver Ingleby[1], Susan Girdwood[1], Alan R. Cookson[1], Russell M. Morphew[1] and David E. Whitworth[1]

1. Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, SY23 3DD, United Kingdom. 2. Department of Biomedical Sciences, Cardiff Metropolitan University, Western Avenue
Cardiff, CF5 2YB, United Kingdom.

Address correspondence to Dr David Whitworth, dew@aber.ac.uk

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Running Title: Eight novel *Corallococcus* species.

Abstract

Corallococcus spp. are common soil-dwelling organisms which kill and consume prey microbes through the secretion of antimicrobial substances. Two species of *Corallococcus* have been described previously (*Corallococcus coralloides* and *Corallococcus exiguus*).

A polyphasic approach was taken to characterise antimicrobial, biochemical and phenotypic properties of eight *Corallococcus* spp. strains and the two type strains. We also report here the genome sequence of the *C. exiguus* type strain (DSM 14696^T).

The genomes of the eight candidate strains, *C. exiguus* DSM 14696^T and *C. coralloides* DSM 2259^T, had an average nucleotide identity below 95% and digital DNA-DNA hybridisation scores less than the 70% lower bound for species identity, indicating they belong to distinct species.

All ten strains, including the two type strains, were thoroughly characterised, including biochemical analysis of their fatty acid methyl esters, substrate utilisation and

sugar assimilation. Each strain gave a distinct profile of properties, which together with their genomic differences supports the proposal of the eight candidate strains as novel species: *Corallococcus exercitus* sp. nov. (AB043A^T = DSM 108849^T = NBRC 113887^T), *Corallococcus interemptor* sp. nov. (AB047A^T = DSM 108843^T = NBRC 113888^T), *Corallococcus aberystwythensis* sp. nov. (AB050A^T = DSM 108846^T = NBRC 114019^T), *Corallococcus praedator* sp. nov. (CA031B^T = DSM 108841^T = NBRC 113889^T), *Corallococcus sicarius* sp. nov. (CA040B^T = DSM 108850^T = NBRC 113890^T), *Corallococcus carmarthenensis* sp. nov. (CA043D^T = DSM 108842^T = NBRC 113891^T), *Corallococcus llansteffanensis* sp. nov. (CA051B^T = DSM 108844^T = NBRC 114100^T) and *Corallococcus terminator* sp. nov. (CA054A^T = DSM 108848^T = NBRC 113892^T).

Importance

Corallococcus is a genus of 'wolf-pack' predators with broad prey ranges and whose genomes contain large numbers of biosynthetic gene clusters for secondary metabolite production. Eight *Corallococcus spp. s*trains were thoroughly characterised using phylogenetic and phylogenomic analyses, growth assays, microscopic imaging, biochemical activity assays, fatty acid profiling, predatory activity assays and antibiotic resistance profiling. The strains exhibited distinct patterns of drug resistance, which mirrored their possession of diverse sets of biosynthetic genes. Multiple metrics confirmed that each strain belonged to a novel species within the *Corallococcus* genus.

Taxonomic assignment of environmental isolates to novel species allows us to begin to characterise the diversity and evolution of members of this biotechnologically important bacterium, which is important as it can guide bioprospecting efforts for novel biologically active metabolites and antimicrobials.

Introduction

Myxobacteria are virtually ubiquitous deltaproteobacteria commonly found in temperate topsoil (Dawid, 2000). Their lifestyle is unusual amongst bacteria, with populations cooperatively responding to starvation by forming multicellular fruiting bodies containing spores, while vegetative growth is supported by their predation of a broad range of prey organisms (Morgan et al., 2010; Livingstone et al., 2017). Myxobacterial predators secrete antimicrobial substances, causing the lysis of prey organisms and release of their

nutrients into the environment, which has led to the controversial assumption that predation, like fruiting, is also cooperative (Marshall and Whitworth, 2019).

Little work has been published that associates myxobacterial taxa with particular ecological roles/niches beyond labelling them as terrestrial or marine, or defining whether or not they degrade cellulose. It has become apparent that individual myxobacterial species can exhibit a great deal of phenotypic variation even within a very small geographical area (Vos and Velicer, 2008; Vos and Velicer, 2009), which may explain our current lack of ecological understanding.

Such phenotypic diversity, which often does not correlate with taxonomy, has also hampered traditional taxonomic approaches to classify the myxobacteria, leading to the adoption of polyphasic approaches for taxonomic assignment (Mohr et al., 2018). Over recent decades myxobacterial classification approaches have focussed on morphological features (including the architecture of colonies and fruiting bodies), biochemical properties and the sequence of conserved genes (particularly the 16S gene). With the advent of whole genome sequencing, it has also become possible to undertake genome-based assessments of bacterial taxonomy and evolution (Chun et al., 2018).

The genus *Corallococcus* was validly described in 2007 as a member of suborder Cystobacterineae within family Myxococcaceae and included three species: *C. coralloides*, *C. exiguus* and *C. macrosporus* (Euzéby, 2007). Subsequently, Lang and Stackebrandt (2009) published an emended description of the genera *Corallococcus* and *Myxococcus*, reassigning *C. macrosporus* to the *Myxococcus* genus as *Myxococcus macrosporus*.

Corallococcus spp. cells are Gram-negative bacilli when growing vegetatively, but produce coral-shaped orange/peach coloured fruiting bodies upon starvation (Garcia et al., 2010). It is one of the myxobacterial genera most easily isolated from soil and exhibits a wide range of predatory activity against diverse microbes (Mohr et al., 2016; Livingstone et al., 2017). This is largely attributable to the secondary metabolites they produce, which possess a range of antimicrobial properties (Xiao et al., 2011; Landwehr et al., 2016).

We have recently completed a comparative genome analysis of 23 *Corallococcus* spp. strains which suggested the sequenced organisms belonged to ten discrete genomospecies, of which eight were likely to be novel (Livingstone et al., 2018). In the current study we undertook a traditional polyphasic characterisation of representatives from each of the seven *Corallococcus* genomospecies. Biochemical and physiological

measurements confirmed that they belong to distinct species and we therefore propose eight novel *Corallococcus* species.

Methods and Materials

Bacterial strains

Strains AB043A, AB047A, AB050A, CA031B, CA040B, CA043D, CA051B and CA054A were originally isolated from soil and identified as *Corallococcus* spp. in a previous study (Livingstone et al., 2017). The type strains of *C. coralloides* (DSM 2259^T) and *C. exiguus* (DSM 14696^T) were obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures). All ten strains were cultured on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar) for further characterisation.

Phenotypic characterisation

Growth properties were assessed at various temperatures (at pH 7.8) and pH values (at 30 ^oC) on VY-2 agar. The biochemical properties of strains were characterised using the API 20E kit (BioMérieux) according to the manufacturer's kit instructions. Stokes' method for antibiotic susceptibility testing was used, with results compared against those for *Escherichia coli* ATCC 25922 and interpreted as 'resistant' or 'susceptible' according to standard rules (BSAC, 1991).

FAME analysis

Saponification of cellular fatty acids was achieved by incubating in 1.875 M NaOH at 100 ^oC for 30 minutes. For methylation 2 volumes of 3.25 M HCl in methanol were added and incubated at 80 ^oC for 10 minutes. Fatty acid methyl esters (FAMEs) were extracted in 1:1 hexane: methyl tert-butyl ether and washed in 0.3 M NaOH (Sasser, 2006). Analysis of FAMEs was performed using an Agilent 7890B gas chromatograph with a Leco Pegasus BT time-of-flight mass spectrometer. The GC was equipped with a CP-Sil 88 capillary column (Agilent CP7489, 100 m x 0.25 mm x 0.2 µm). The carrier gas was helium at a constant pressure of 20.7 psi and flow rate of 0.7 ml/min. The GC oven start temperature was 70 °C, ramping at 8 °C/min to 100 °C, then at 5 °C/min up to 170 °C for 10 minutes before a final ramp at 4° C/min to 240 °C for 30 minutes. The inlet and transfer line to the MS were both 240 °C. A split injection (1:50) of 1 µl sample was used.

Data were analysed using the ChromaTOF software from Leco. Samples were compared to standard solutions run under the same conditions and peaks identified by both

retention time and mass spectra. The standards used were: 37 component FAME mix (Supelco, CRM47885), Linoleic acid, conjugated methyl ester (Supelco, O5362), Mixture ME93 (Larodan, 90-1093) and Bacterial Acid Methyl Esters CP Mixture (Matreya, 1114). The data presented are mean values derived from biological replicates (prepared on at least two occasions from at least two cultures).

Predation assays

The predatory activity of the eight candidate strains has been previously reported (Livingstone et al., 2017). For comparison, here the predatory profile of strains *C. coralloides* DSM 2259^T and *C. exiguus* DSM 14696^T were assayed using the same protocol, which involved inoculation onto lawns of ten prey organisms (see Livingstone et al., 2017 for details of prey strains). The diameter of the predatory zone on the prey lawn was measured after seven days' incubation at 30 ^oC.

Genome analysis

Draft genome sequences of the eight candidate strains were published previously (Livingstone et al., 2018) while the *C. coralloides* DSM 2259^T genome was downloaded from the NCBI database. *C. exiguus* DSM 14696^T was sequenced in this study using 2 × 250 bp paired-end reads on the Illumina Hiseq 2500 platform by MicrobesNG (Birmingham, United Kingdom). The raw reads were subjected to Kraken 2 for read mapping, BWA-MEM for quality control and SPAdes 3.7 for *de novo* assembly (Li and Durbin, 2009; Bankevich et al., 2012; Wood and Salzberg, 2014). The DSM 14696^T genome sequence is available from the NCBI nucleotides database under BioProject accession PRJNA547735.

Complete 16S rRNA gene sequences were extracted from the genomes for phylogenetic analysis and similarity searches. Neighbour-joining trees were constructed in MEGA-7.0 (Kumar et al., 2016), using the Kimura 2-parameter model with 500 boostraps. Phylogenomic relationships were analysed using AMPHORA2 based on 31 concatenated marker genes and visualised as a maximum likelihood tree with 500 bootstraps generated using a Jones-Taylor-Thornton model in MEGA 7.0 (Kerepesi et al 2014; Kumar et al., 2016). The average nucleotide identity (ANI) and digital-DNA/DNA hybridization (dDDH) were calculated using the genome-to-genome distance calculator (Meier-Kolthoff, 2013). The comprehensive antibiotic resistance database (CARD) was used to identify antibiotic resistance genes in the genomes and to correlate them with the phenotypic antibiotic susceptibility tests (Jia et al., 2017).

Results

The eight candidate strains represent eight novel genomospecies.

The genome sequence of *C. coralloides* DSM 2259^T has previously been shown to lie within a different genomospecies to the eight candidate strains, whether considering ANI or dDDH values (Livingstone et al., 2018). However, the lack of a *C. exiguus* genome sequence meant that it was possible that one of the candidate genomospecies could have been *C. exiguus*.

A draft genome sequence of *C. exiguus* DSM 14696^T was therefore generated. Similarly to other *Corallococcus spp.* draft genomes, the DSM 14696^T assembly had a total size of 10,463,210 bp spread over 880 contigs with 9122 coding sequences and a GC content of 69.5%. The N50 and L50 values for the genome sequence were 24,436 bp and 136 respectively (ie. the largest 136 contigs together constituted more than half of the genome sequence, and the 136th contig was 24.4 Kbp long).

The genome sequences of DSM 14696^T, DSM 2259^T and the eight candidate strains were compared in every pair-wise combination and ANI and dDDH scores calculated (Table 1). In all pair-wise comparisons the ten strains had ANI scores between 84% and 92% with dDDH scores of 50% or less. Based on the currently accepted boundaries for defining same-species membership at 95% for ANI and 70% for dDDH (Chun et al., 2018) this indicates they belong to ten separate species within the same genus.

Previous genome sequence comparisons (Livingstone et al., 2018) have demonstrated that *Corallococcus* spp. genomospecies lay in two large phylogenomic groups (A and B), with *C. coralloides* found within Group A. Performing comparisons of the *C. exiguus* DSM 14696^T genome with other published *Corallococcus* spp. genomes revealed that it also lies within Group A, within the genomospecies composed of strains AB004, AB018, AB030, AB38B and CA041A described by Livingstone et al. (2018), which can therefore now be identified as strains of *C. exiguus*.

Phylogenetic relationships between the ten genomospecies

Evolutionary relationships between the ten strains were visualised by generating phylogenetic trees based on 16S rRNA gene sequences (Figure 1A), concatenated sequences of 31 conserved genes (Figure 1B), and ANI values (Figure 1C). The 16S rRNA tree suggests four groupings, which is mirrored wth ANI and dDDH values. *C. exiguus* and *C. coralloides* lie

within the same group, alongside AB047A. The four Group B strains are found together as a pair of pairs (CA031B/CA054A with CA040B/CA051B) and the remaining three Group A strains clustering together (AB043A/AB050A/CA043D).

Surprisingly, strains CA031B and CA054A show little difference in 16S sequence (Figure 1A) yet the Amphora tree based on 31 conserved genes, demonstrated substantial differences between strains (Figure 1B) as did the ANI-derived tree (Figure 1C).

Physiology and biochemical characterisation

The cells of all strains were Gram-negative bacilli measuring approximately 0.5-1.0 µm by 3.0-7.0 µm, which looked morphologically similar in scanning electron micrographs to the representative strains shown; CA051B and AB043A (Figure 2). Colonies exhibited swarming growth on VY-2 and were of a pale orange/peach colour, with darker fruiting bodies. Testing for growth at different temperatures (at pH 7.8) demonstrated all strains grew at a temperature of 30 ^{o}C, no growth of strains at 37 ^{o}C or above, while strain growth at 35 ^{o}C was strain dependent (Table 2). Of note were strains AB043A and CA043 which grew unusually well at 35 ^{o}C for myxobacteria. The pH-dependence of strain growth was also tested (at 30 ^{o}C). The optimum pH was 7 or higher for all strains with only four of the strains exhibiting growth at pH 5 (Table 2). AB050A exhibited an exceptionally restricted pH tolerance, only growing at pH 7-7.8. Only strains CA040B and CA054A exhibited the same profile of pH and temperature-dependent growth (Table 2).

The ability to metabolise a variety of carbon sources was tested and all strains found to be incapable of metabolising adipate, arabinose, arginine, caprate, gluconate, mannitol, mannose, N-acetyl glucosamine or urea. However some strains were able to metabolise citrate, esculin, gelatin, glucose, malate, maltose, *o*-nitrophenyl-β-D galactopyranoside and phenyl acetate (Table 3). Only strains CA040B and DSM 2259^{T} exhibited the same profile of substrate utilisation (Table 3). Fatty acid methyl ester (FAME) analysis was also employed to characterise the fatty acids of each strain (Table 4). The profile of fatty acid derivatives detected was unique to each strain, with CA040B possessing a particularly diverse set of lipids.

Predatory activity and antibiotic resistance profiling

Myxobacteria are known to possess predatory activity against a broad range of prey organisms. The predatory activity of the candidate strains and DSM 2259^{T} have already been described (Livingstone et al., 2018), but for this study we assessed the activity of DSM

14696^T for comparison (Table 5). DSM 14696^T is on average the best predator of the ten strains, out-predating all other predator strains on seven of the ten prey organisms, while DSM 2259^T demonstrated the lowest average predatory activity.

Myxobacterial predation is thought to involve, at least partly, the secretion of cocktails of antibiotic secondary metabolites encoded by large biosynthetic gene clusters (BGCs). The BGCs which direct antibiotic production, and their associated resistance genes, are members of the accessory pan-genome and each strain/taxon possesses a distinctive set of BGCs. This is presumably responsible for the individuality of predatory activities against diverse prey (Livingstone et al., 2018). We therefore characterised the antibiotic resistance profile of the ten *Corallococcus* spp. strains to investigate the individuality of antibiotic production by each strains.

All isolates were resistant to Ampicillin, Ceftazidime, Ertapenem and Piperacillin/Tazobactam and all were susceptible to Amikacin, Ciprofloxacin and Trimethoprim/Sulfamethoxazole (Table 6). CA031B was unique in being sensitive to Imipenem, while CA043D was uniquely sensitive to Gentamicin and Cefotaxime. CA031B, CA040B and CA054A were sensitive to Augmentin whereas all other strains were resistant (Table 6). The Resfinder tool of the comprehensive antibiotic resistance database (CARD) demonstrated the presence of the multidrug resistance efflux gene *adeF* within the genomes of each strain. In addition, CA031B, CA040B and CA051B were found to possess the AAC(3)-lllb gene for aminoglycoside resistance, and AB043A possesses the gene for the MsbA multidrug resistance transporter (Table 6).

Proposal of eight novel Corallococcus *species.*

On the basis of genomic and phylogenetic differences, distinct growth characteristics, biochemical activities, fatty acid profiles and antibiotic resistance profiles, we propose that the eight candidate strains described here each belong to and typify novel *Corallococcus*: *Corallococcus exercitus* sp. nov. (AB043A^T), *Corallococcus interemptor* sp. nov. (AB047A^T), *Corallococcus aberystwythensis* sp. nov. (AB050A^T), *Corallococcus praedator* sp. nov. (CA031B^T), *Corallococcus sicarius* sp. nov. (CA040B^T), *Corallococcus carmarthensis* sp. nov. (CA043D^T), *Corallococcus llansteffanensis* sp. nov. (CA051B^T) and *Corallococcus terminator* sp. nov. (CA054A^T).

This proposal also allows to define species membership for additional *Corallococcus* spp. isolates described previously (Livingstone et al., 2018), on the basis of ANI values ≥95%.

Specifically, AB011P, AB045, CA049B and CA054B are classified as *C. coralloides*, AB004, AB018, AB030, AB032C, AB038B and CA041A as *C. exiguus*, AB049A and AB050B as *C. interemptor*, and CA031C and CA047B as *C. praedator*.

Discussion

The genus Corallococcus

The Myxococcaceae family currently contains five validly described genera; *Aggregicoccus*, *Corallococcus*, *Myxococcus*, *Pyxidicoccus* and *Simulacricoccus*. When compared to the genus *Myxococcus*, the remaining four genera are relatively poorly understood with *Corallococcus* being the second best characterised. Indeed, *Myxococcus xanthus* is the single best studied myxobacterium, being the model organism for the whole Myxococcales order. In June 2019, a Pubmed search for '*Myxococcus xanthus*' gave 1,469 hits, while the search term '*Corallococcus*' gave a mere 45. Compared to the five valid *Myxococcus* species (*Myxococcus fulvus, Myxococcus macrosporus, Myxococcus stipitatus, Myxococcus virescens* and *M. xanthus*), only two have been described in *Corallococcus* (*C. coralloides* and *C. exiguus*).

Corallococcus spp. members are nevertheless important organisms in their own right and deserve to emerge from under the shadow of *Myxococcus* spp. They are amongst the most abundant myxobacterial genera isolated from soils (Mohr et al., 2016; Livingstone et al., 2017), and are proven producers of antimicrobial metabolites (Xiao et al., 2011; Landwehr et al., 2016). Genome sequencing of 23 *Corallococcus* isolates revealed an open pan-genome with strains having highly individual complements of BGCs, suggesting that the few compounds isolated from *Corallococcus* spp. to date represent just the tip of an iceberg of novel *Corallococcus* metabolites (Livingstone et al., 2018; Gregory et al., 2019).

Diversity within the Corallococcus genus

When originally isolated, and subjected to 16S rRNA gene sequencing, the eight candidate *Corallococcus* spp. strains described here exhibited greatest 16S sequence similarity to *Corallococcus coralloides/exiguus*, with similarities of at least 98.7 %. 16S rRNA phylogenetic analysis demonstrated that the strains belonged to a clade distinct from the other Myxococcaceae genera, encompassing *C. coralloides* and *C. exiguus* (Livingstone et al., 2017). This mirrored the results of a study in 2005, which concluded that 'the genus

Corallococcus may embrace a broad range of yet-to-be described novel species.' (Stackebrandt and Päuker, 2005).

In recent years, it has become increasingly appreciated that 16S rRNA phylogenetic taxonomic assignment has limitations, which can be overcome by considering more genes, ultimately considering every gene in an organism's genome (phylogenomics). The gold standard for sequence-based taxonomic assignment has therefore shifted from 16S rRNA to genome-based methods (Richter and Rosselló-Móra, 2009), and has recently culminated in the proposal of new standards for genome-based taxonomy based on 'overall genome relatedness indices' (OGRIs) such as ANI (Chun et al., 2018).

Following assessment of the genomic diversity of our *Corallococcus* spp. culture collection, OGRIs indicated that our 23 sequenced strains belonged to at least ten discrete genomospecies, one of which included the *C. coralloides* type strain (Livingstone et al., 2018). Sequencing the *C. exiguus* type strain genome in this study has now allowed us to identify a second genomospecies as being *C. exiguus*, confirming that *C. exiguus* and *C. coralloides* are discrete species despite their virtually identical 16S rRNA gene sequences (Stackebrandt and Päuker, 2005).

Corallococcus Physiology

Early taxonomic classification of the myxobacteria relied heavily on fruiting body morphology, colony behaviour and colouration. However, it became clear that such phenotypes were difficult to reproduce, being sensitive to laboratory conditions such as batch variations in media and adaptation of lineages to repeated sub-culturing. Therefore, myxobacterial taxonomists incorporated gene sequence based approaches and polyphasic assessments of physiology and biochemical properties (Garcia et al., 2010; Mohr et al., 2018). Rather than relying solely on genome-based arguments to assess taxonomy, we have instead tried to reconcile a traditional polyphasic approach with genome-based taxonomic approaches in this study.

No consistent morphological differences were observed between candidate strains at a colonial or cellular level. Physiological differences were observed, for instance in preferred growth temperatures and pH, but these were often slight (Table 2). All strains were capable predators, as expected given their initial isolation using *E. coli* bait, yet significant variation was observed in their predatory efficiencies and antibiotic resistance profiles. *C. exiguus* was a particularly efficient predator, while *C. coralloides* was particularly

poor (Table 6). Differences in antibiotic susceptibility were observed between candidate strains, but with many strains showing identical susceptibility profiles. Phenotypic distinctiveness of candidate strains was most apparent in biochemical tests for compound metabolism (Table 3) and fatty acid profiling (Table 4) with each strain having an absolutely unique profile.

Given their similarities to *Corallococcus* type strains, and their distinctive biochemical, predatory and genomic characteristics, we are confident in proposing that the eight candidate strains characterised above, each typifies a novel *Corallococcus* species, which we describe below. Given that characterising 23 *Corallococcus* isolates has here led to the identification of ten novel species, we suggest that the statement of Stackebrandt and Päuker (2005) remains true, that 'the genus *Corallococcus* may [still] embrace a broad range of yet-to-be described novel species.'

Summary

Here we present morphological, physiological, predatory, biochemical and genomic data which support the designation of eight strains as type strains for novel species within the genus *Corallococcus*. As well as being untapped reservoirs of novel metabolites, these species will be extremely useful in investigating the evolution, diversity and physiology of antimicrobial microbes.

Species Descriptions

Description of *Corallococcus aberystwythensis* sp. nov.

Corallococcus aberystwythensis (ab.e'r.yst.w'yth.en.sis N.L. masc. adj. *aberystwythensis* from Aberystwyth, reflecting the fact that the new species was isolated near Aberystwyth in Wales [52.41°N 4.08°W]).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 1.2-1.4 µm x 3.0-7.0 µm under the light microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^{o}C but not 35 ^{o}C and at pH 7.0-7.8. Produces indole, nonanoate, decanoate, undecanoate, tetradecanoate, pentadecanoate, palmitoleate, oleate, iso-C13:0, iso-C15:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp. Cells prey upon *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, Bacillus subtilis,* and *Candida albicans*. DNA GC content is 70.0 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026036). Phylogenetically most similar to *C. exercitus* and *C. carmarthensis.*

The type strain (AB050A^{T} = DSM 108846^{T} = NBRC 114019^{T}) was isolated from soil collected from a field near Aberystwyth, United Kingdom [gridref 52.41°N 4.08°W].

Description of *Corallococcus carmarthensis* sp. nov.

Corallococcus carmarthensis (car.ma'r.th.en.sis. N.L. masc. adj. c*armarthensis* from Carmarthen, the fact that the new species was isolated near Carmarthen, Wales [51.86°N 4.31°W]).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 1.1-1.5 µm x 3.0-5.0 µm under the light microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30-35 ^{o}C but not at 37 ^{o}C, and at pH 5.0-9.0. Assimilates citrate and phenyl-acetate, hydrolyses esculin, gelatine and *o*-nitrophenyl-β-D-galactopyranoside. Produces nonanoate, decanoate, undecanoate, dodecanoate, tetradecanoate, pentadecanoate, hexadecanoic,

palmitoleate, iso-C13:0, iso-C15:0, iso-C16:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp., except sensitive to Cefotaxime and Gentamicin. Cells prey upon *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Candida albicans*, and feed particularly well on *Klebsiella pneumoniae, Enterococcus faecalis* and *Bacillus subtilis*. DNA GC content is 69.9 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026042). Phylogenetically most similar to *C. exercitus* and *C. aberystwythensis.*

The type strain (CA043D^T = DSM 108842^T = NBRC 113891^T) was isolated from soil collected from a field near Carmarthen, United Kingdom [gridref 51.86°N 4.31°W].

Description of *Corallococcus exercitus* sp. nov.

Corallococcus exercitus (ex.e'r.cit.us. L. nom. n. *exercitus* the army, reflecting concerted killing of prey by a large number of cells).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 0.4-0.5 µm x 2.8-5.6 µm under the scanning electron microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^oC but not 35 ^oC and at pH 5.0-9.0. Hydrolyses gelatine and assimilates glucose. Produces indole, decanoate, undecanoate, pentadecanoate, heptadecanoiate, palmitoleate, 7,10-hexadecenoate, linolenate, iso-C13:0, iso-C15:0, iso-C16:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp. Cells prey upon *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, Bacillus subtilis,* and *Candida albicans*. DNA GC content is 70.3 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026050). Phylogenetically most similar to *C. aberystwythensis* and *C. carmarthensis.*

The type strain (AB043A^T = DSM 108849^T = NBRC 113887^T) was isolated from soil collected from a field near the village of Goginan, United Kingdom [gridref 52.41°N 3.93°W].

Description of *Corallococcus interemptor* sp. nov.

Corallococcus interemptor (int.er.e'mpt.or. L. nom. n. *interemptor* the destroyer, reflecting the destruction of neighbouring prey cells).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 1.2-1.3 µm x 3.0-6.0 µm under the light microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^oC but not 35 ^oC and at pH 5.0-9.0. Reduces nitrate and hydrolyses esculin, gelatine and *o*-nitrophenyl-β-D-galactopyranoside. Produces indole, nonanoate, decanoate, undecanoate, tridecanoate, pentadecanoate, palmitoleate, 2-hexyl-cyclopropaneoctanoate, iso-C13:0, iso-C15:0, iso-C16:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp. Cells prey upon *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, Candida albicans*, and grows particularly well on *Bacillus subtilis*. DNA GC content is 69.8 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026034). Phylogenetically most similar to *C. coralloides* and *C. exiguus.*

The type strain (AB047A^T = DSM 108843^T = NBRC 113888^T) was isolated from soil collected from a field near the village of Goginan, United Kingdom [gridref 52.41°N 3.93°W].

Description of *Corallococcus llansteffanensis* sp. nov.

Corallococcus llansteffanensis (llan.stef.an.en.sis. N.L. masc. adj. *llansteffanensis* from Llansteffan, reflecting the fact that the new species was isolated near the village of Llansteffan in Wales [51.77°N 4.39°W]).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, measuring 0.4-0.9 µm x 2.0-4.0 µm under the scanning electron microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^oC but not 35 ^oC and at pH 6.0-9.0. Hydrolyses esculin. Produces indole, nonanoate, undecanoate, decanoate, dodecanoate, tridecanoate, pentadecanoate, palmitoleate, oleate, linoleate, linolenate, 2-hexyl-cyclopropaneoctanoate, iso-C13:0, iso-C15:0, iso-C16:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp. Cells prey upon *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae,*

Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, and Candida albicans. DNA GC content is 70.3 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026045). Phylogenetically most similar to *C. sicarius.*

The type strain (CA051B^T = DSM 108844^T = NBRC 114100^T) was isolated from soil collected from the edge of a stream near the village of Llansteffan, United Kingdom [gridref 51.77°N 4.39°W].

Description of *Corallococcus praedator* sp. nov.

Corallococcus praedator (prae.da't.or. L. nom. n. *praedator* the plunderer, reflecting the acquisition of nutrients from prey cells).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 1.2-1.4 µm x 3.0-6.0 µm under the light microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^oC but not 35 ^oC and at pH 6.0-7.8. Assimilates malate and hydrolyses esculin. Produces indole, nonanoate, decanoate, undecanoate, pentadecanoate, palmitoleate, 2-hexyl-cyclopropaneoctanoate, iso-C13:0, iso-C15:0, iso-C16:0, and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp., except sensitive to Augmentin and Imipenem. Cells prey upon *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, Candida albicans*, but grow poorly on *Pseudomonas aeruginosa*. DNA GC content is 69.6 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026038). Phylogenetically most similar to *C. terminator.*

The type strain (CA031B^T = DSM 108841^T = NBRC 113889^T) was isolated from soil collected from woodland near Tanerdy, United Kingdom [gridref 51.87°N 4.29°W].

Description of *Corallococcus sicarius* sp. nov.

Corallococcus sicarius (si.ca'ri.us. L. nom. n. *sicarius* the killer, reflecting killing of prey during predation).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 1.2-1.4 µm x 3.0-6.0 µm under the light microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^oC but not 35 ^oC and at pH 6.0-9.0. Hydrolyses esculin, gelatine and *o*-nitrophenyl-β-D-galactopyranoside. Produces tridecanoate, pentadecanoate, palmitoleate, iso-C13:0, iso-C15:0, iso-C16:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp., except sensitive to Augmentin. Cells prey upon *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, and Candida albicans*. DNA GC content is 70.2 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026040). Phylogenetically most similar to *C. llansteffanensis.*

The type strain (CA040B^T = DSM 108850^T = NBRC 113890^T) was isolated from soil collected from woodland near Tanerdy, United Kingdom [gridref 51.87°N 4.29°W].

Description of *Corallococcus terminator* sp. nov.

Corallococcus terminator (ter.min.a't.or. L. nom. n. *terminator* the ender, reflecting predatory termination of prey viability).

Vegetative cells are Gram-negative bacilli tapering slightly at the ends, appearing 1.3-1.5 µm x 3.0-7.0 µm under the light microscope. Colonies were swarming and appeared pale orange/peach on VY-2 agar (0.5% dried baker's yeast, 0.1% $CaCl_2.2H_2O$, 1.5% agar). Fruiting bodies were irregular spheroids, yellow/orange in colour. Aerobic growth observed at 30 ^oC but not 35 ^oC and at pH 6.0-9.0. Assimilates maltose and hydrolyses esculin, gelatine and *o*-nitrophenyl-β-D-galactopyranoside. Produces indole, nonanoate, decanoate, tridecanoate, pentadecanoate, palmitoleate, linoleate, linolenate, iso-C13:0, iso-C15:0, iso-C16:0 and iso-C17:0. Antibiotic resistance/susceptibility profile typical for *Corallococcus* spp. Cells prey upon *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus faecalis, and Candida albicans*. DNA GC content is 69.5 mol%. The draft genome sequence of the organism is available from GenBank (Biosample number SAMN10026047). Phylogenetically most similar to *C. praedator.*

497 The type strain (CA054A^T = DSM 108848^T = NBRC 113892^T) was isolated from soil
498 collected from the edge of a stream near the village of Llansteffan, United Kingdom [gridref
499 51.77°N 4.39°W].
500
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505
506 **Author Contributions**
507 PL undertook phenotypic and bioinformatics characterisation of the strains under
508 the supervision of DW and RM, OL and SG performed FAME analysis and AC captured the
509 EM images. DW and PL drafted the manuscript which was edited by all authors.
510

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Figures

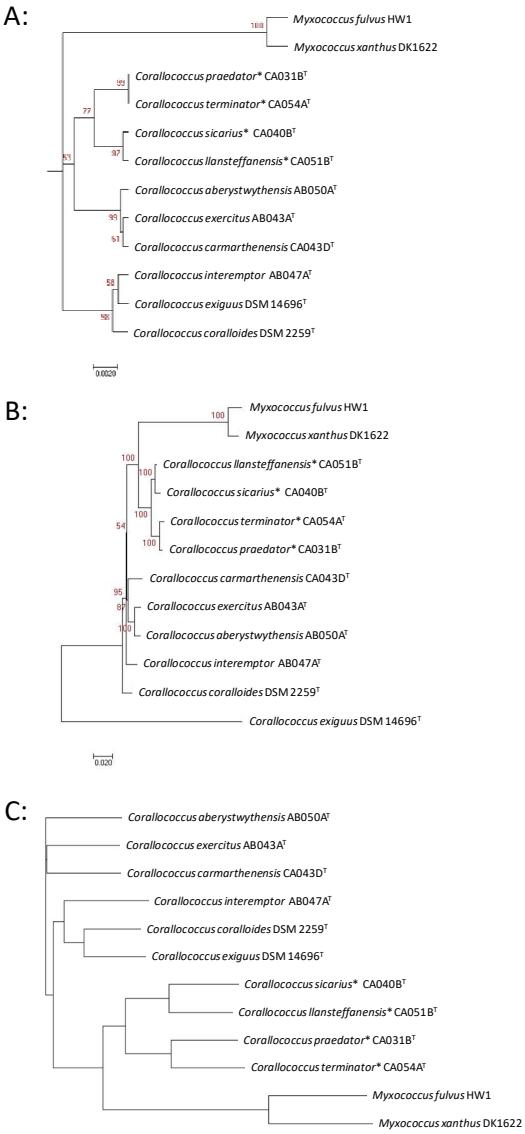
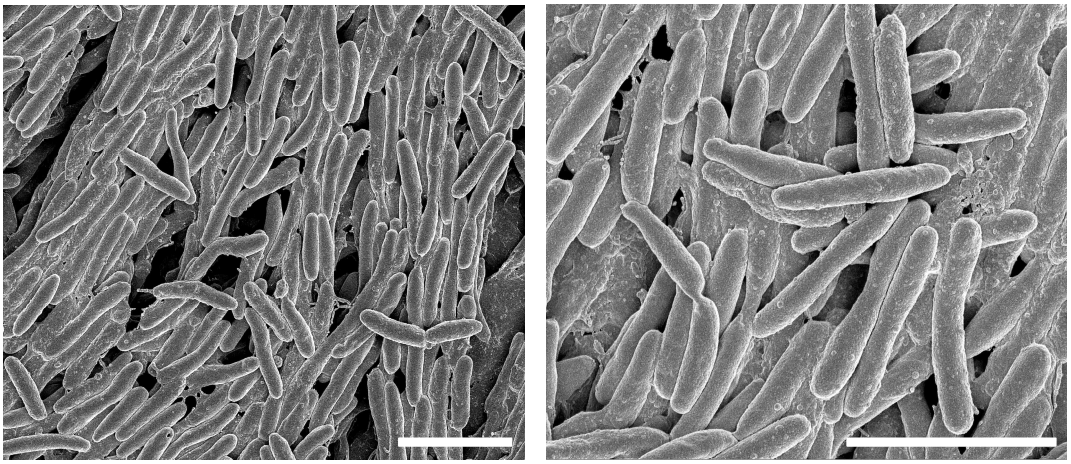


Figure 1. Phylogenetic trees of *Corallococcus spp.* members, with two strains from the sister genus *Myxococcus* for comparison. Strains indicated with * belong to Group B while the others belong to Group A. A: Neighbour-joining 16S rRNA sequence tree. B: Maximum likelihood Amphora tree based on the sequence of 31 conserved genes. C: Neighbour-joining tree constructed using ANI values.

A:



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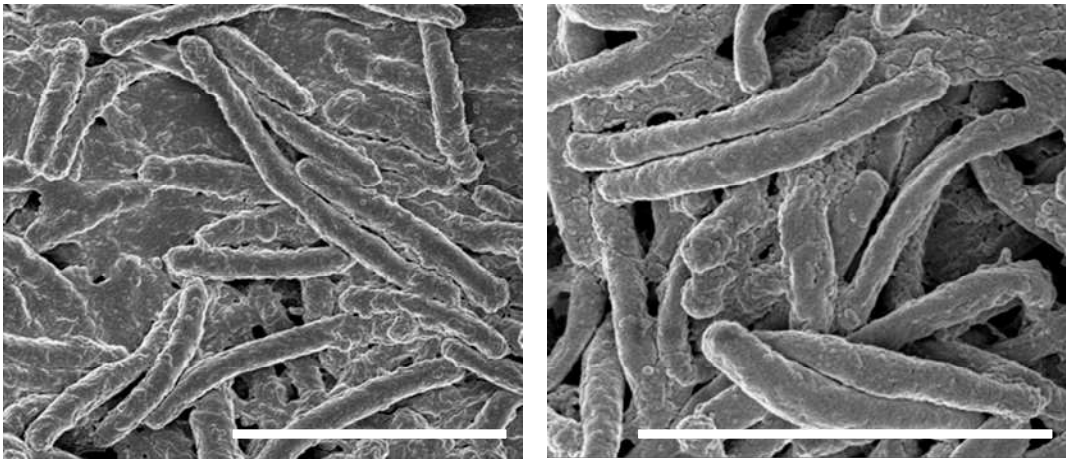

Figure 2. Scanning electron micrographs of *Corallococcus spp*. A: CA051B cells (*C. llansteffanensis*). B: AB043A cells (*C. exercitus*). Bars are 4 µm long.

599 **Tables**

600

dDDH \ ANI	DSM 14696^T	DSM 2259^T	AB047A	AB050A	CA043D	AB043A	CA031B*	CA054A*	CA040B*	CA051B*
DSM 14696^T	100	94	92	91	92	92	87	86	86	87
DSM 2259^T	34	100	92	91	91	92	86	85	86	86
AB047A	44	46	100	90	90	90	85	84	85	85
AB050A	44	44	43	100	91	91	84	84	85	85
CA043D	44	44	42	48	100	91	84	84	86	85
AB043A	43	44	43	47	48	100	84	84	85	85
CA031B*	30	31	30	31	31	32	100	91	88	87
CA054A*	30	30	30	30	31	31	50	100	88	87
CA040B*	30	30	30	31	31	32	36	35	100	92
CA051B*	31	31	31	32	32	33	37	35	50	100

601

602 **Table 1.** ANI and dDDH values for pair-wise comparisons between the eight candidate
603 strains, *C. exiguus* DSM 14696^T and *C. coralloides* DSM 2259^T. ANI values are shown above
604 the diagonal and dDDH values below the diagonal. ANI values above 90% and dDDH values
605 above 40% are shaded grey. Strains indicated with * belong to phylogenetic Group B while
606 the others belong to Group A.

607

608

Table 2. Growth characteristics of *Corallococcus* spp. Rate of growth is indicated as '-' (no growth), '+' (slow), '++' (moderate) or '+++' (fast). Temperature-dependence was tested at pH 7.8, while pH-dependence was tested at 30 °C.

	AB043A	AB047A	AB050A	CA031B	CA040B	CA043D	CA051B	CA054A	DSM 2259^T	DSM 14696^T
30 °C	++	++	+	+	++	+++	++	++	++	+++
35 °C	++	+	-	-	-	++	+	-	+	+
37 °C	-	-	-	-	-	-	-	-	-	-
40 °C	-	-	-	-	-	-	-	-	-	-
pH 5.0	+	+	-	-	-	+	-	-	-	+
pH 6.0	++	+	-	+	+	+	+	+	++	++
pH 7.0	+++	++	+	+	++	+++	++	++	++	+++
pH 7.8	++	++	+	+	++	+++	++	++	++	+++
pH 8.0	++	++	-	-	++	+++	++	++	+++	+++
pH 9.0	++	++	-	-	++	+++	++	++	+++	+

Table 3. Metabolic activity of *Corallococcus* spp. Activity is either '-' (inactive) or '+' (active) as tested by the API method (BioMérieux).

	AB043A	AB047A	AB050A	CA031B	CA040B	CA043D	CA051B	CA054A	DSM 2259^T	DSM 14696^T
Citrate assimilation	-	-	-	-	-	+	-	-	-	+
Esculin hydrolysis	-	+	-	+	+	+	+	+	+	-
Gelatine hydrolysis	+	+	-	-	+	+	-	+	+	+
Glucose assimilation	+	-	-	-	-	-	-	-	-	-
Indole production	+	+	+	+	-	-	+	+	-	+
Malate assimilation	-	-	-	+	-	-	-	-	-	-
Maltose assimilation	-	-	-	-	-	-	-	+	-	+
Nitrate reduction	-	+	-	-	-	-	-	-	-	-
Phenyl-acetate assimilation	-	-	-	-	-	+	-	-	-	-
o-Nitrophenyl-β-D galactopyranoside hydrolysis	-	+	-	-	+	+	-	+	+	+

Table 4. Fatty acids methyl esters detected in *Corallococcus* spp. A '-' denotes a relative abundance of less than 1 % of total FAMEs, and a '+' denotes presence (at least 1 % of total FAMEs) in each strain. FAMEs are shaded light grey if they represent more than 5 % of a strain's FAMEs and dark grey if more than 10 %.

	AB043A	AB047A	AB050A	CA031B	CA040B	CA043D	CA051B	CA054A	DSM 2259^T	DSM 14696^T
Nonanoic acid, methyl ester (C9:0)	-	+	+	+	-	+	+	+	+	+
Decanoic acid, methyl ester (C10:0)	+	+	+	+	-	+	+	+	+	+
Undecanoic acid, methyl ester (C11:0)	+	+	+	+	-	+	+	-	+	+
Dodecanoic acid, methyl ester (C12:0)	-	-	-	-	-	-	+	-	+	+
Tridecanoic acid, methyl ester (C13:0)	-	+	-	-	+	-	+	+	+	+
11-Methyl-dodecanoic acid, methyl ester (iso-tridecanoic) (iso C13:0)	+	+	+	+	+	+	+	+	+	+
Tetradecanoic acid, methyl ester (Myristic) (C14:0)	-	-	+	-	-	+	-	-	-	-
12-Methyl-tridecanoic acid, methyl ester (iso-tetradecanoic) (iso C14:0)	-	-	-	-	-	-	-	-	-	+
Pentadecanoic acid, methyl ester (C15:0)	+	+	+	+	+	+	+	+	+	+
13-Methyl-tetradecanoic acid, methyl ester (iso-C15:0)	+	+	+	+	+	+	+	+	+	+
Hexadecanoic acid, methyl ester (C16:0)	-	-	-	-	-	+	-	-	+	+
14-Methyl-pentadecanoic acid, methyl ester (iso-C16:0)	+	+	-	+	+	+	+	+	+	+
9-Hexadecenoic acid (Z), methyl ester (Palmitoleic) (C16:1)	+	+	+	+	+	+	+	+	+	+
7,10-Hexadecadienoic acid, methyl ester (C16:2)	+	-	-	-	-	-	-	+	+	+
Heptadecanoic acid, methyl ester (C17:0)	+	-	-	-	-	-	-	-	+	+
2-Hexyl-cyclopropaneoctanoic acid,	-	+	-	+	-	-	+	-	-	+

methyl ester (C17:0)										
15-Methyl-hexadecanoic acid, methyl ester (Isomargaric) (iso-C17:0)	+	+	+	+	+	+	+	+	+	+
Octadecanoic acid, methyl ester (Stearic) (C18:0)	-	-	-	-	-	-	-	-	-	+
9-Octadecenoic acid (Z), methyl ester (Oleic) (C18:1)	-	-	+	-	-	-	+	-	-	-
9,12-Octadecadienoic acid (E,E), methyl ester (C18:2)	-	-	-	-	-	-	-	-	-	-
9,12-Octadecadienoic acid (Z,Z), methyl ester (Linoleic) (C18:2)	-	-	-	-	-	-	+	+	-	+
Octadecatrienoic acid, methyl ester (Linolenic) (C18:3)	+	-	-	-	-	-	+	+	+	+
Tetracosanoic acid, methyl ester (C24:0)	-	-	-	-	-	-	-	-	-	+

Table 5. Predatory activity of *Corallococcus* spp. Values denote the diameter (mm) of zones of killing against ten prey organisms after seven days' growth at 30 ^{o}C.

	AB043A	AB047A	AB050A	CA031B	CA040B	CA043D	CA051B	CA054A	DSM 2259^{T}	DSM 14696^{T}
Escherichia coli	30	30	20	31	25	33	26	23	25	54
Pseudomonas aeruginosa	20	19	22	9	25	30	20	17	30	43
Klebsiella pneumoniae	43	35	31	36	35	45	30	29	6	40
Proteus mirabilis	34	31	29	28	33	35	29	23	19	46
Staphylococcus aureus	20	22	16	23	19	35	25	23	27	62
Staphylococcus epidermidis	26	32	20	38	24	38	28	24	12	48
Staphylococcus saprophyticus	20	23	17	25	22	22	28	16	15	46
Enterococcus faecalis	19	25	17	19	22	29	21	18	15	27
Bacillus subtilis	31	40	24	31	28	43	35	24	6	21
Candida albicans	33	36	28	45	42	44	38	26	6	49

Table 6. Antibiotic resistance profiles of *Corallococcus* spp. A: observed resistance to antibiotics in plate assays ('+' denotes resistant, '-' denotes sensitive). B: Presence of resistance genes in the genome ('+' denotes presence, '-' denotes absence).

A:	AB043A	AB047A	AB050A	CA031B	CA040B	CA043D	CA051B	CA054A	DSM 2259^T	DSM 14696^T
Amikacin	-	-	-	-	-	-	-	-	-	-
Ampicillin	+	+	+	+	+	+	+	+	+	+
Augmentin	+	+	+	-	-	+	+	-	+	+
Cefotaxime	+	+	+	+	+	-	+	+	+	+
Ceftazidime	+	+	+	+	+	+	+	+	+	+
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-
Ertapenem	+	+	+	+	+	+	+	+	+	+
Gentamicin	+	+	+	+	+	-	+	+	+	+
Imipenem	+	+	+	-	+	+	+	+	+	+
Trimethoprim/Sulfamethoxazole	-	-	-	-	-	-	-	-	-	-
Piperacillin/Tazobactam	+	+	+	+	+	+	+	+	+	+
B:	AB043A	AB047A	AB050A	CA031B	CA040B	CA043D	CA051B	CA054A	DSM 2259^T	DSM 14696^T
adeF	+	+	+	+	+	+	+	+	+	+
AAC(3)-IIIb	-	-	-	+	+	-	+	-	-	-
msbA	+	-	-	-	-	-	-	-	-	-